

EXHIBIT 16

7

Consecutive digestion of APTag/BglII & HindIII

BglII digestion 19 ul

water 17 ul

React I 2 ul

HindIII 2 ul

40 ul at 37°C

(purify by elutip method)

05:00-06

digestion of 4-1BB PCR products

4-1BB (#3, #4) 16 ul

React 3 2 ul

spermidine 1 ul

EcoRI 1 ul
20 ul

4-1BB (5, 3') 16 ul

React 3 2 ul

spermidine 1 ul

BglII 1 ul
20 ul

~~Phenol/chloroform ext. (no heat)~~ at 37°C 05:00-06

add React 1 2 ul

water 16 ul

StuI 2 ul
40 ul

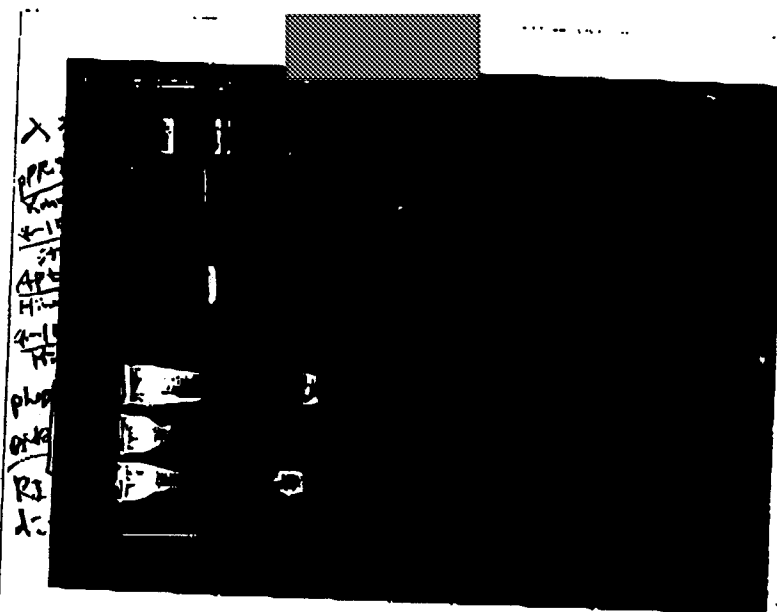
React 1 2 ul

water 16 ul

HindIII 2 ul
40 ul

05:45

(2)



Ligation

	Exp.	Control (left)
PPR998/XmH2-R2 (25 ng/ul)	4.0 ul	4.0 ul
4-1B3 PCR/STH2-R2 (12 ng/ul)	0.5 ul	—
5X buffer	2.0 ul	2.0 ul
Water	3.0	3.5
Ligase	0.5 ul	0.5
	10.0 ul	10.0

Legation

APtag / Hind III - Bgl II (150ng/ul)

exp.

self

1 ul

1 ul

4-1BB (PCR) / Hind III - Bgl II (8ng/ul)

2 ul

—

5X buffer

2 ul

2 ul

water

4.5

6.5

ligase

0.5

0.5 ul

10 ul

100 ul

metrol (ul)

4.0 ul

—

2.0 ul

3.5

0.5

10.0

